REMARKS

USPTO Office Communication of July 9, 2008

The Office Communication sent on July 9, 2008 stated that the amendment to the claims filed on March 17, 2008 did not comply with the requirements of 37 CFR 1.121(c). Applicants have revised the Amendment submitted on March 17, 2008, and attest that the Amendment in Response submitted herewith now complies with 37 CFR 1.121(c). Applicants have removed the text of canceled claim 2, and have inserted claims 4, 6-11, 17, and 18, as amended by the Preliminary Amendment of October 21, 2005. The remaining text of the Amendment of March 17, 2008 has not been changed. Applicants therefore respectfully request that this Amendment in Response be considered.

Claim Status

Claims 1-18 are pending. Applicants have canceled claim 2 without prejudice to pursue the subject matter in a future application. Claims 1, 3, 12, 14, 15 and 16 have been amended.

Support for the amendments to the claims

Support for amended claim 1 may be found *inter alia* on page 17 of the Specification, lines 15-27; claim 1 and claim 2 as filed.

Support for amended claim 3 may be found *inter alia* on page 17 of the Specification, lines 21-24.

Support for amended claim 12 may be found *inter alia* on page 5 of the Specification, line 10.

Support for amended claims 13-16 may be found *inter alia* in claims 13-16 as filed.

Applicants respectfully request the entry of this Amendment. Upon entry, Claims 1, 3-18 are pending and under examination.

Rejections Under 35 U.S.C. §112

Claims 12-13 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The rejection is respectfully traversed.

Claim 12 has been amended to recite that the tissue animal cells are brain neurons. Claim 13 has been amended to clarify that the aim is to express the sequences in an animal cell. Applicants submit that claim 12 and 13 have been amended to obviate the rejection. Accordingly, Applicants respectfully request that the rejection of claims 12-13 under 35 U.S.C. §112, second paragraph, be withdrawn.

Rejections Under 35 U.S.C. §102

Claims 1-4 and 6-15 are rejected under 35 U.S.C. §102(e) as being anticipated by Chtarto et al. The rejection is respectfully traversed.

Chtarto et al. is directed exclusively to a vector comprising a bi-directional antibiotic controlled activator-responsive promoter. In particular, the promoter comprises a tetracycline (Tet) responsive element.

The **Tet responsive promoter** is a synthetic sequence composed of 7 repetitions of an 8 nucleotide-long **prokaryotic sequence.** Such promoter is not able to exploit the endogenous mammalian transcriptional machinery in order to work properly. Therefore, to render this promoter functional, it is necessary to express an **additional** transgene encoding for a chimeric transcriptional activator composed of two halves, the first one being of

prokaryotic origin and the second one being of **viral or human** origin.

A fundamental feature of the tetracycline-responsive expression system is that the promoters used must be insulated from nearby competing enhancers in order to prevent inappropriate transactivation and preserve integrity of modulation by doxycycline. Therefore, only minimal promoters have been so far used in this system. Moreover, the Tet-regulated system depends on the expression (or exogenous administration) of transactivators, whose expression (or administration) may encounter problems in vivo.

In contrast, the present invention is directed to **synthetic eukaryotic bidirectional promoters**, based on the juxtaposition of a core promoter element placed upstream and in opposite orientation to an efficient promoter, that exploits the endogenous transcriptional machinery available to most animal cells types to drive robust expression of two divergent transcripts. The fact that the bidirectional promoter of the present invention comprises a minimal **viral** promoter **and** a **full length animal** promoter represents a fundamental difference over the prior art in general, and over Chtarto et al. in particular.

It is well known in this art that the eukaryotic and prokaryotes promoters act differently. For example,

> *The promoter contains specific DNA sequences, response elements, that are recognized by proteins known as transcription factors. These factors bind to the promoter sequences, recruiting RNA polymerase, the enzyme that synthesizes the RNA from the coding region of the gene.*
>
> - *In prokaryotes, the promoter is recognized by RNA polymerase and an associated sigma factor, which in turn are brought to the promoter DNA by an activator protein binding to its own DNA sequence nearby.*

- *In eukaryotes, the process is more complicated, and at least seven different factors are necessary for the transcription of an RNA polymerase II promoter."*

See **Exhibit A, 5 pages.**

In addition, it is well known from general biology text books that a promoter of a prokaryote organism cannot work in the environment of an eukaryote organism and vice versa.

The bidirectional promoter of the present invention can be based on **any eukaryotic promoter**: ubiquitous, constitutive, tissue specific or endogenously regulated.

In nature, few instances of bidirectional promoters have been documented and only very recently recent surveys of the human genome have indicated an abundance of divergently transcribed gene pairs representing more than 10% of the human genome, whose transcriptional start sites are separated by less than 1 kb. In addition, it has been suggested that more than half of the human promoters does not exhibit strong directionality in transcript initiation and can function in a bidirectional fashion. Thus, the synthetic bidirectional promoters of the present invention may mimic a well-represented and evolutionary conserved feature of eukaryotic transcription, providing a structural basis for their robust performance.

More specifically, Chtarto et al. require the use of a transactivator factor encoded by a reverse antibiotic controlled transactivator nucleotide sequence for activating the bi-directional promoter. *See*, for instance, Chtarto et al. column 5, lines 12-16:

> *In said construct or system the bi-directional antibiotic controller activator responsive promoter/operator sequence 4 is advantageously activated by the transactivator factor 7, encoded by the reverse antibiotic controlled transactivator 7, encoded by the reverse*

antibiotic controlled transactivator nucleotide sequence 6 in the presence of said antibiotic 5.

That Chtarto et al. is only directed to an antibiotic inducible/repressible genetic construct is clear throughout the document. *See* further Fig. 6, column 4, lines 42-43, column 5, lines 22-32.

The present invention offers the construction of a bidirectional promoter comprising a minimal viral promoter and a full length eukaryotic promoter. *See*, for instance, description page 7, lines 3-8:

A bidirectional promoter made by minimal core promoter elements from the human cytomegalovirus (mCMV) joined upstream, and in opposite orientation, to an efficient promoter, derived from the human phosphoglycerate kinase (PGK) or poly-ubiquitin UBI-C gene, was driving divergent transcription of two RNAs.

In conclusion, Chtarto et al. do not anticipate the present invention because Charto et al. do not teach each and every aspect of the present invention. Accordingly, Applicants respectfully request that the rejection of claims 1-4 and 6-15 under 35 U.S.C. §102(e) be withdrawn.

Claims 1-4 and 6-11 and 13-18 are rejected under 35 U.S.C. §102(e) as being anticipated by Itoh et al. The rejection is respectfully traversed.

Itoh et al. is directed exclusively to a vector comprising a low molecular weight compound-responsive bidirectional promoter and a DNA encoding a low molecular weight compound-controlled transactivator. In particular, the low molecular weight compound is tetracycline or doxycycline and the low molecular weight

compound-controlled transactivator is a reverse tetracycline transactivator.

Therefore, arguments presented above concerning Chtarto et al. are also valid in respect to Itoh et al.

In conclusion, Itoh et al. do not anticipate the present invention because Itoh et al. do not teach each and every aspect of the present invention. Accordingly, Applicants respectfully request that the rejection of claims 1-4 and 6-11 and 13-18 under 35 U.S.C. §102(e) be withdrawn.

Claims 1-4, 7-8, 10 and 14 are rejected under 35 U.S.C. §102(b) as being anticipated by Fux et al. The rejection is respectfully traversed.

Fux et al. discloses the construction of two vectors, PDuoRex7 and pDuoRex8 (*see* page 114, left column, last paragraph) which contain two antibiotic-responsive expression units in divergent orientation. Such pDuoRex-based dual regulated expression requires concomitant production of tTA and PIT (*see* page 114, right column, first paragraph).

Thus, arguments presented above concerning Chtarto et al. are also valid in respect to Fux et al.

In conclusion, Fux et al. do not anticipate the present invention because Fux et al. do not teach each and every aspect of the present invention. Accordingly, Applicants respectfully request that the rejection of claims 1-4, 7-8, 10 and 14 under 35 U.S.C. §102(b) be withdrawn.

It should be noted that none of the cited prior art documents discloses a bidirectional promoter comprising a minimal viral promoter and a full length animal promoter. Therefore none of the cited documents anticipate the present invention.

Moreover, starting from the teaching of Chtarto et al., or Itoh et al. or Fux et al. and due to the evolutionary distance between prokaryotes and mammalians and to their differences in the transcriptional machinery, the person skilled in the art would not predict and foresee that mammalian promoters could be also exploited for building a bidirectional promoter. Therefore, the invention is not obvious in respect to such cited prior art documents.

Rejections Under 35 U.S.C. §103

Claim 5 is rejected under 35 U.S.C. §103(a) as being unpatentable over Chtarto et al. or Itoh et al., in view of Hope et al. (US 6,136,597).

Chtarto et al. and Itoh et al. have been discussed above. Hope et al. disclose an RNA export element which mediates efficient transport of RNA from the nucleus to the cytoplasm. This RNA export element is referred to as WPRE. WPRE is a post transcriptional regulatory element. Hence, Hope et al. teach using WPRE to increase transgene expression.

Therefore, even though assuming it is appropriate to combine Chtarto et al., and Hope et al., or Itoh et al., and Hope et al., (which the Applicants do not concede), such combination would not teach or suggest all of the features of dependent claim 5. In particular, the combined teaching of Chtarto, Itoh and Hope does not teach or suggest constructing a bidirectional promoter comprising a minimal viral promoter and a full length animal promoter.

Accordingly, Applicants respectfully request that the rejection of claim 5 under 35 U.S.C. §103(a) be withdrawn.

For the Examiner's information, the corresponding **European Application** of this subject application has been granted and is attached hereto as **Exhibit B, 71 pages**.

Conclusion

In summary, Applicants believe that all grounds of rejections have been addressed and earnestly request the Examiner to place this application in condition for allowance.

If a telephone interview would be of assistance in advancing prosecution of the subject application, Applicants' undersigned attorney invites the Examiner to telephone him at the number provided below. If any other fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 50-1891.

Respectfully submitted,

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